INFLUENCE OF SOIL MOISTURE AND SULFUR BACTERIA ON SULFUR OXIDATION IN FOUR KANSAS SOILS

by

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INTRODUCTION

All crops need sulfur in varying amounts. It has been known for some time that plants utilize sulfur in the form of sulfates. As time goes on certain soils are becoming deficient in sulfur, and consequently sulfur will have to be added to these soils if normal yields are to be maintained. It is known that sulfur is added through rain in the form of sulfates. This addition is greater near areas of large population than in rural districts. Sulfur is also added in the form of sulfates, although not intentionally, in certain fertilizers.

It has been found useful to add elemental sulfur to some alkaline soils to increase the availability of plant nutrients and increase the hydrogen ion concentration. Sulfur also may be added to neutral and acid soils as a nutrient. The elemental sulfur is oxidized by the sulfur bacteria to sulfur dioxide, and then again to sulfur trioxide. The sulfur trioxide then combines with water in the soil to form sulfuric acid.

Soils vary in their power to oxidize sulfur. In western Kansas some soils show a marked ability to oxidize sulfur while some do not. Environmental conditions of the soil are thought to have a marked effect on the rate of sulfur oxidation. Moisture content, temperature, reaction, composition, texture, aeration, bacterial content and general nutrient balance of the soil are among the factors that may play a major

part in the determination of the sulfofying power of a specific soil. No careful study has been made of the effect of moisture upon the amount of sulfur oxidized. Therefore an experiment was designed to determine the effects of moisture upon the amount of sulfur oxidized in four Kansas soils.

Soil moisture content is a factor that influences the growth of higher plants as well as microorganisms. The relative availability to higher plants of soil water at different moisture contents within the available water range has been a subject of study by a number of investigators. Some studies have indicated that moisture is equally available over the entire range from the wilting point to field capacity. Other studies have shown that plant growth decreases as the tension of water in the soil becomes greater. It is not possible to maintain a constant soil moisture content while higher plants are growing. The use of sulfur bacteria as an indicator of the relative availability of soil moisture at different tensions offers a means of approaching this problem.

REVIEW OF LITERATURE

It has been previously stated that some soils are deficient in sulfur. This element may be added to soils either to supply a nutrient need or to counteract alkalinity. Soils of Alabama, Maryland and Oklahoma (12) are examples of soils requiring nutrient sulfur, while Arizona (22), Oregon (25) and

Alberta (24) soils are examples of soils that may be benefited by the acidifying properties of elemental sulfur.

The Oregon Station (26) found that an application of sulfur at the rate of one ton per acre was adequate for a period of four years. Repeated applications caused only the slightest drop in the hydrogen ion concentration. Hibbard (15) and Lint (20) claimed that the increase in acidity of the soil was caused by the oxidation of sulfur. Small applications; i.e., one ton per acre, according to the Arizona Experiment Station (22), are oxidized very rapidly, in fact at optimum moisture and temperature the process is completed within three weeks. The results of Lint (20) bear this point out in that he found little change in acidity after the seventh week. Heavier applications (22), nevertheless, have a more pronounced and prolonged effect.

Stephenson (37) found that the rate of sulfur oxidation is dependent upon the degree of fineness of the fertilizer material. Kappen and Quensell's (17) results agreed with this finding. However, McGeorge and Green (22) claim that the coarser grained material will produce as good an oxidation as will the finer, more expensive grades.

Halverson and Bollen (14) found that the addition of sulfur to a soil tended to increase the oxidizing power. These authors also showed that a correlation existed between sulfur oxidizing power of a soil and the sulfate content of that soil. Neller (23) and Haynes (13) showed that sulfur was

oxidized more rapidly under alkaline conditions than acid, and that the concentration of salts did not have a detrimental effect upon oxidation. According to McGeorge and Green (22) sulfur reduces alkalinity, improves the physical condition, and increases the available calcium, potassium, phosphate and carbon dioxide.

Brown and Kellog (7) were the first workers to make a thorough study of sulfofication. They showed that oxidation was primarily brought about by bacterial action, although some chemical oxidation does exist. Kappen and Quensell (17) obtained similar results and pointed out that oxidation may be a result, in part, of chemical reactions. Aquino (2, 3) found that sulfur added to sterilized soils does not undergo oxidation. Shedd (35) stated that inoculated soils undergo more rapid oxidation than uninoculated soils. Brown and Johnson (6) showed that each soil has a definite sulfur oxidizing capacity of its own.

Environmental conditions also affect the oxidation of sulfur (6) and actually these environmental factors are the same as the ones that affect the growth and viability of bacteria. Kaluzhskii (16) emphasized the importance of temperature. He stated that the quantities of water soluble sulfates vary in the soil throughout the year, maximum amounts occurring in the spring with the minimum in the fall. From this point of view the oxidation of sulfur follows closely that of nitrogen. Kaluzhskii (16) also found that an increase in

temperature, up to a certain point, brought about an increase in bacterial activity. Shedd (35) discovered that by increasing the air content of the soil to 50 percent, the sulfofication increased accordingly, but if it were increased even more, the oxidation would decrease. This author also found that the period of incubation should not be less than two weeks in order to accurately determine the sulfofying power of a soil. The Oregon Station (27, 28) states that the temperature and moisture of the soil appear to be the most important factors governing the rate of sulfur oxidation, although sulfur oxidation is very strong even in air dry soils.

Veihmeyer and Henrickson (39) have concluded that water is available to higher plants over the entire range from field capacity to the wilting point. Various authors, however, have indicated that water is not equally available over the entire range down to the wilting point. Magness (21) found that the rate of apple growth decreased when the driest part of the root zone approached the wilting point even though most of the root zone was considerably wetter. Lewis (19) found that the rate of growth of pears was considerably retarded when the soil moisture was reduced to 70 percent of the available moisture. Davis (9), in his work with young corn plants, found that water appeared less available for growth when the moisture content of the soil decreased only 3 percent below field capacity and growth ceased while 3 percent of the available water remained in the soil.

Starkey (36) states that three bacteria are responsible for oxidation of sulfur in the soil. All of these organisms are capable of oxidizing sulfur in the elemental form. Probably the most important of the three is Thiobacillus thiooxidans. This organism is capable of living at a pH of 0.5 (5). It oxidizes sulfur more rapidly than the other two. Its optimum growth occurs at room temperature. This organism is rarely found in cultivated soils to which no sulfur has been added. However, it may be found in rather large amounts in soils that have been enriched with sulfur. Thiobacillus thioparus is probably the slowest oxidizer of the three. Optimum activity and growth occur at a neutral pH. Both Thiobacillus thiooxidans and Thiobacillus thioparus are aerobic, autotrophic bacteria.

The third, Thiobacillus denitrificans is a facultative anaerobic autotrophic bacterium that oxidizes thiosulfate to sulfate, although according to Waksman (40) it is capable of oxidizing elemental sulfur. Under anaerobic conditions this organism uses nitrate as the hydrogen acceptor which is reduced to nitrogen. The natural habitat of these organisms is the soil. Although other types of soil flora may oxidize sulfur, their activity cannot be compared to the amount produced when these three bacteria are present.

EXPERIMENTAL METHODS

Soils Used

In this experiment four soils were used, two from western Kansas and two from the eastern portion. Carlson (8) found that the two soils from western Kansas differed in their ability to oxidize sulfur in the field. Ulysses silt loam caused the higher sulfur oxidation of the two soils used. Ulysses silt loam belongs to the Ulysses series which includes Chestnut soils that have developed in the drier parts of the Chestnut soils zone on loess-mantled uplands. These soils are between the Keith and Colby soils in character. They are darker in color than the Colby surface layer and lighter than the Keith soils. The soils are often limy at the surface in cultivated areas while in the grassed areas they may not be calcareous above the horizon of lime carbonate enrichment.

The profile of the Ulysses silt loam consists of four divisions. The A_1 horizon ranges in thickness from 6 to 10 inches and is a pale brown to very dark greyish brown friable silt loam. The B_2 horizon ranges in thickness from 8 to 10 inches and is greyish brown to dark brown in color and slightly more compact than the A_1 layer. It may be of the friable silt loam or silty clay loam type. The B_3 layer ranges in thickness from 5 to 8 inches and is a very pale brown to brown, friable calcareous silt loam. The last horizon, C, may be

several feet in depth and is a white to very pale brown calcareous loess.

The second soil used in this study was Fort Collins loam. Fort Collins loam belongs to the Fort Collins series which includes Brown soils that have been developed from calcareous alluvial terrace deposits of medium to moderately fine texture. This series is characterized by greyish brown surface layers and friable lighter colored subsoils with fairly well-developed horizons of lime carbonate accumulation. The subsurface is more silty and coherent in nature than those of the Greeley soils. Parent material and substrata of this series are stratified alluvium as contrasted to the uniform silty loessial parent material of the otherwise somewhat similar soils of the Colby series. These soils are intermediate in character between the Big Horn and Rocky Ford soils. The surface layers of the Fort Collins series are darker than the Rocky Ford and lighter in color than the Big Horn soils.

The profile of the Fort Collins loam consists of five horizons. The first ranges in thickness from 2 to 6 inches and is a greyish brown friable loam or fine sandy loam. The second layer ranges in thickness from 4 to 10 inches and is a pale brown friable loam of ill defined prismatic structure. It is commonly calcareous and is firmer in position than the first layer. The third horizon ranges in thickness from 12 to 16 inches and is a very pale brown, friable, cloddy calcareous silt loam. It also may be a very fine sandy loam or

a light textured clay loam. The fourth layer is characterized by a very pale brown friable massive or light silt loam. This layer ranges in thickness from 15 to 20 inches and is calcareous but less so than the lower portion of the above area. The last horizon is characterized by a very pale brown color. It is a friable calcareous silty clay loam or loam.

The cation exchange capacity of the two soils from western Kansas was found by treating them first with 1N potassium acetate and then treating with 1N ammonium acetate in order to displace the exchangeable potassium (30). The potassium content of the solution was then determined on a Perkin-Elmer Model 52A flame photometer by the internal standard method. Extractable sodium was determined by extracting the soil with an ammonium acetate solution and then determining the amount of sodium with the flame photometer. Soluble sodium was determined with the flame photometer in a solution that was extracted from a saturated soil paste. The exchangeable sodium was found by subtracting the soluble sodium from the extractable sodium. The electrical conductivity of this saturated extract was determined with a conductivity bridge (38).

The pH was determined with a glass electrode pH meter. The mechanical analyses of the two soils from western Kansas was determined by the hydrometer method (4). The total nitrogen content of both soils was determined by the Kjeldahl method. The analyses of Ulysses silt loam and Fort Collins loam may be compared in Table 1. Moisture tension curves

of the four soils are given in Fig. 1. It may be noted that the curves for the four soils differ widely and that the soils show large differences in their moisture retention characteristics.

No analyses were made of the two soils from eastern Kansas. These two soils came from the Kaw Valley and are believed to have been formed from alluvial deposits. As yet these soils have not been classified. However, the clay soil is thought to lie between a clay loam and a silty clay and the sand is believed to be between a very fine loamy sand and a fine loamy sand.

Table 1. Analyses of Ulysses silt loam and Fort Collins loam.

Analyses	Ulysses silt loam	Fort Collins loam
pH	7.53	8.03
Percent total nitrogen	0.11	0.08
Original sulfate content	0.00	0.00
Cation exchange capacity (m.e./100 gm)	21.12	16.92
Extractable sodium (m.e./100 gm)	00.45	00.63
Soluble sodium (m.e./100 gm)	00.38	00.18
Exchangeable sodium (m.e./100 gm)	00.08	00.46
Exchangeable sodium percent	00.35	2.70
Elect. cond. (millimohs/cm)	00.57	2.60
Percent sand	26.0	40.8
Percent silt	54.0	44.8
Percent clay	20.0	14.4

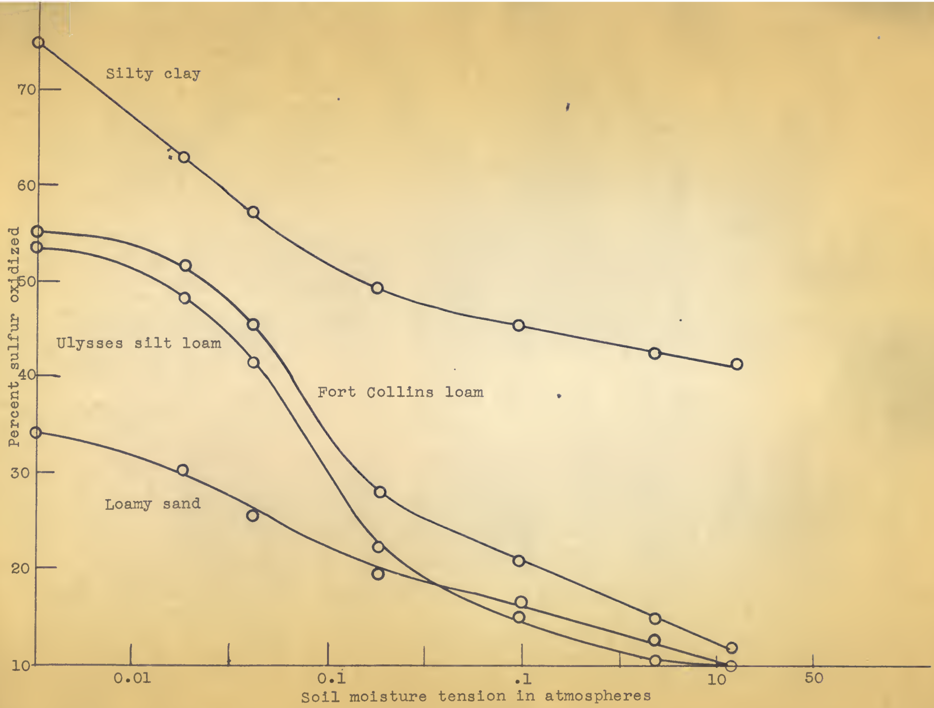

Fig. 1. Soil moisture tension curves of four soils.

Sulfur Oxidation Studies

Flours of sulfur were added to all soils at the rate of four thousand pounds per acre or two-tenths of a gram per hundred grams of soil. The sulfur was sifted through a hundred mesh sieve before use.

In order to prepare sulfur treated soils at various moisture content, 10 to 20 gram samples were placed in rubber rings (2 in. diameter x $1\frac{1}{2}$ in. deep) and soaked overnight. All samples were run in triplicate. The Leamer and Shaw (18) tension table was used to adjust the soil to 30 and 60 centimeter tensions. A porous ceramic plate placed in a pressure cooker, operating on the principle of the pressure plate apparatus of Richards and Fireman (33) and Richards (34) was used to obtain one third atmosphere and one atmosphere tensions. The Richards (31, 32) pressure-membrane apparatus was used for 5 and 15 atmospheres.

As it was not feasible to use the pressure-membrane apparatus for 30 atmospheres, the soil was adjusted to 15 atmospheres tension and allowed to dry in order to attempt to obtain the moisture retention that would occur at 30 atmospheres. To determine whether or not sulfur could be oxidized under air dry conditions, 20 gram samples of air dry soil were used. To determine whether or not sulfur could be oxidized under anaerobic conditions, samples were set up with a one to one ratio of soil

to water. In this case, 20 grams of soil were mixed with 20 cubic centimeters of distilled water. Samples were also adjusted to zero tensions by using a tension table (18).

Once the samples had been adjusted to the tensions mentioned, the moisture content of each sample was obtained. The samples were then placed in 125 cc Erlenmeyer flasks and stoppered. These flasks were placed in closed cans that had two inches of water at the bottom. These cans were then placed in a constant temperature room with a temperature of $25^{\circ} \pm 2^{\circ}$ C. The samples of Ulysses and Fort Collins soils were incubated for 3, 6, 9, and 12 week periods while the other two soils were incubated only 3 weeks. The flasks were opened every other day in order to insure aeration.

At the end of every three weeks the moisture content, pH and sulfate content of the soil samples were determined. In attempting to determine the amount of sulfur oxidized in a soil, a modified procedure outlined by the U. S. Regional Salinity Laboratory (38) was used. Due to these changes it is necessary to outline the procedure used. As it was not feasible to weigh the samples at the onset of the sulfate determinations, the moisture content was taken and the flasks were weighed with the samples and then again without, thus making it possible to determine the original weight on an oven dry basis. Thirty-three ml of a 1N neutral solution of ammonium acetate were added directly to the samples in the flasks in which they were incubated. The samples were then agitated

on a mechanical shaker for 15 minutes. The shaken samples were transferred to 50 ml centrifuge tubes and centrifuged at 2000 r.p.m. for 5 minutes. The clear supernatant liquid was decanted into 100 ml volumetric flasks. The extractions were repeated twice and the volume was made up to 100 ml.

A 5 ml aliquot of the solution was transferred to a 12 ml centrifuge tube. To this aliquot two drops of bromphenol blue (0.04 percent in a 95 percent ethanol solution) were added and 1N hydrochloric acid was added until the previously purple solution became yellow. The samples were then placed in the refrigerator for a period of 10 minutes. Two ml of the precipitating agent benzidine hydrochloride solution (consisting of four grams of the reagent, 7 ml 1N hydrochloric acid and enough water to make 250 ml) were added to the samples. The samples were agitated slightly to hasten precipitation and then put into the refrigerator for one hour. The samples were then centrifuged at the rate of 3000 r.p.m. for 10 minutes. The supernatant fluid was pipetted off by means of suction. The precipitate and walls of the tube were washed with 5 ml of 95 percent acetone. This procedure was repeated twice.

The acetone-washed precipitate was washed into a 30 ml beaker with 10 ml of water from a wash bottle. To this solution 0.2 ml of a 0.05 percent solution of phenol red was added. The samples were then titrated boiling hot with 0.01N sodium hydroxide solution. The end point in this case was taken as a very light pink color. With each set of samples blanks were

run in order to correct for variations in the solutions used.

Moisture contents were determined gravimetrically. The dry samples were diluted with an equivalent weight of water for the pH determinations.

Bacterial Identifications

As the Fort Collins and Ulysses soils used in this experiment showed a marked difference in their ability to oxidize sulfur, it was found necessary to determine the type and amounts of sulfur bacteria present in both soils in order to attempt to discover the possible reason for their deviation. Four types of media were used: thiosulfate agar (1), thiosulfate medium (liquid) (1), motility agar (11) and Trautwein's medium (1). The thiosulfate agar medium (1) was used as a diagnostic medium for the aerobic bacteria, *Thiobacillus thiooxidans* and *Thiobacillus thioparus*. The Trautwein's medium (1) was used as a differential medium for the isolation of *Thiobacillus denitrificans* under anaerobic conditions. This organism is capable of anaerobic existence if there are nitrates in the medium. It also grows aerobically, but for the purpose of identification this method seemed more feasible, as the other two organisms cannot survive anaerobic conditions.

It has been stated previously that *Thiobacillus thiooxidans* is more easily found in soils that have been enriched with

sulfur. In fact it is almost impossible to find it in cultivated soils that have not had an addition of sulfur. Therefore, it appeared desirable to divide the samples into soil containing sulfur and soil without sulfur. Twenty samples per soil, 10 with sulfur and 10 without sulfur were spread over petri dishes containing the medium previously designated and incubated aerobically at room temperature, as this temperature is optimum for both the aerobic organisms. Ten samples of soil were incubated anaerobically at room temperature, again with and without sulfur using Trautwein's medium (1). All samples were incubated for a period of two weeks and checked for growth every day. Those colonies that appeared to be gram negative rod shaped cells were inoculated into motility agar. If they proved to be motile they were inoculated into thiosulfate liquid medium (1).

Thiobacillus thioparus produces a pellicle consisting of cells and free sulfur in the liquid medium. On thiosulfate agar this bacterium has colonies that are small circular and whitish yellow due to the precipitated sulfur. Thiobacillus thiooxidans shows scant growth with nearly transparent colonies on thiosulfate agar. On thiosulfate medium this bacterium produces a uniform turbidity. The medium also becomes considerably acid and sulfur is precipitated. The pH of this medium was checked every three days for two weeks which was the incubation period in the liquid medium. Thiobacillus denitrificans has colonies that are thin or weakly opalescent

on Trautwein's medium. In thiosulfate liquid medium, this bacterium produces a large amount of nitrogen gas and therefore those colonies that were thought to belong to this genus, and perhaps this species, were inoculated into fermentation tubes containing this medium. By the above described methods these organisms were identified and isolated.

It was desirable to find out how many sulfur bacteria there were per gram of soil. Samples containing sulfur and no sulfur were used. The plate method (40) consists of diluting the soil with distilled sterile water, making series of dilutions, so that 1 ml of the final dilution will allow colonies to develop on the plate. The dilutions ranged from 1:10 to 1:10,000. One ml of each was placed into a sterile petri dish. Thiosulfate agar (1) was then poured into the plates. The plates were incubated at room temperature for two weeks. All the colonies were checked by the same procedure mentioned above and counts were made accordingly.

EXPERIMENTAL RESULTS

Effect of Soil Moisture Tension upon Percent Sulfur Oxidized and Change in pH Due to Sulfur

The oxidation of sulfur in soil results in an increase in acidity, an increase in the amount of sulfate, and a disappearance of elementary sulfur.

It may be noticed in Tables 2 and 3 that three moisture contents were used for the Ulysses and Fort Collins soils that were not determined by the various mechanisms discussed in the previous section. The first with a moisture content of approximately 2 percent denotes the air dry condition. The second, approximately 8 percent denotes the drier than 15 atmospheres condition. The last is a 1:1 ratio of soil to water.

Tables 2, 3, 4, and 5 give data on moisture contents, pH values, and sulfur oxidation values for the four soils at various moisture tensions and incubation periods. The percent moisture at the beginning and end of the incubation times differs slightly. Probably, when a loss occurred, evaporation took place and perhaps when a gain was observed some organic process occurred which resulted in an increase in the moisture content. However, in either case the difference was never large enough to warrant a change in the given tension.

The Ulysses silt loam and the Fort Collins loam, as seen in Tables 2 and 3, differ rather widely in their ability to oxidize sulfur. Carlson (8) confirmed this point in the field. It should be mentioned that the tension the soils were subjected to in no way affect the bacteria (29). As the time of incubation increased, it may be noticed that the amount of sulfur oxidized also increased. The maximum amount of oxidation of the Ulysses silt loam occurred, at all incubation periods, at 0.029 atmospheres. In the Fort Collins soil the maximum amount of oxidation for the most part, occurred at a

Table 2. Sulfur oxidation and pH in Ulysses silt loam after 3, 6, 9, and 12 week incubation periods at various soil moisture levels.

Soil moisture tension in atm.	Percent moisture at start	Percent moisture at end	M.E. of sulfate /100 gms soil	Percent sulfur oxidized	pH
			After 3 weeks' incubation		
-	2.17	2.18	0.42	3.45	7.40
-	8.00	8.10	0.49	4.00	7.30
15	10.79	10.00	-	-	-
5	11.13	10.98	0.25	2.06	-
1	15.88	15.80	2.73	22.34	6.20
0.333	22.69	22.24	3.61	29.57	5.99
0.059	41.61	41.82	5.70	46.74	5.50
0.029	48.19	48.20	6.79	55.61	5.25
0.000	53.29	53.14	1.15	9.40	6.80
-	100.00	99.98	0.98	8.07	6.92
			After 6 weeks' incubation		
-	2.15	2.19	1.43	11.76	6.75
-	8.00	7.95	1.49	12.20	6.67
15	10.85	10.72	1.89	15.48	6.56
5	11.37	11.00	4.02	32.96	-
1	15.84	15.33	5.80	47.51	5.32
0.333	27.40	26.98	5.97	48.96	5.20
0.059	41.60	41.51	6.56	53.74	5.01
0.029	48.17	47.90	9.43	77.33	4.81
0.000	53.20	53.28	1.91	15.63	6.51
-	100.00	99.98	1.23	10.06	6.72
			After 9 weeks' incubation		
-	2.16	2.15	1.84	15.08	6.41
-	8.00	8.00	2.60	21.31	6.00
15	10.63	10.30	3.47	28.43	5.90
5	11.28	10.97	5.64	46.20	5.51
1	15.84	15.20	11.91	97.70	4.29
0.333	22.83	32.37	12.02	98.51	4.04
0.059	41.77	41.25	12.16	99.69	4.03
0.029	47.70	47.50	12.19	99.95	4.01
0.000	53.20	53.21	2.59	21.19	5.71
-	100.00	99.66	1.57	12.90	6.70
			After 12 weeks' incubation		
-	2.00	2.20	2.63	21.59	5.96
-	8.00	8.37	3.01	24.66	6.02
15	11.00	10.84	4.97	40.76	5.31
5	11.40	11.44	8.13	66.66	5.20
1	15.76	15.82	12.20	100.00	4.01
0.333	22.63	22.62	12.20	100.00	4.00
0.059	42.21	42.19	12.20	99.99	4.00
0.029	47.72	47.63	12.20	99.99	4.02
0.000	53.20	53.13	3.07	25.15	5.29
-	100.00	99.98	1.94	15.92	6.41

Table 3. Sulfur oxidation and pH in Fort Collins loam after 3, 6, 9, and 12 week incubation periods at various soil moisture levels.

Soil moisture tension in atm.	Percent moisture at start	Percent moisture at end	M.E. of sulfate /100 gms soil	Percent sulfur oxidized	pH
			After 3 weeks' incubation		
-	3.13	3.11	-	-	8.03
-	9.33	9.00	-	-	8.00
15	12.55	12.00	-	-	-
5	15.38	15.00	-	-	-
1	20.85	20.47	0.07	0.56	7.96
0.333	27.38	26.98	2.14	17.23	7.21
0.059	46.63	45.98	3.25	28.43	6.30
0.029	53.76	53.50	2.21	18.11	7.00
0.000	54.30	54.08	0.97	7.99	7.51
-	100.00	99.00	0.65	5.33	7.80
			After 6 weeks' incubation		
-	3.14	3.18	0.15	1.22	7.99
-	9.33	9.05	-	-	7.94
15	12.63	12.63	-	-	7.85
5	15.19	15.00	0.02	0.13	-
1	20.89	20.29	3.32	27.24	6.32
0.333	27.40	27.05	4.63	38.80	6.00
0.059	46.58	45.98	5.61	46.07	5.70
0.029	53.59	52.99	5.98	48.96	5.61
0.000	54.30	54.15	1.66	13.64	7.02
-	100.00	99.85	0.87	0.87	7.45
			After 9 weeks' incubation		
-	3.13	3.15	0.65	5.27	7.02
-	9.33	9.23	1.11	9.08	6.99
15	12.74	12.35	1.65	13.52	6.89
5	15.17	15.05	2.73	22.35	6.70
1	20.56	20.07	7.09	58.08	6.21
0.333	27.46	27.38	7.82	64.09	5.41
0.059	46.48	46.00	8.47	69.36	4.98
0.029	52.81	52.90	6.88	56.43	5.01
0.000	54.30	54.15	2.26	18.51	6.50
-	100.00	100.00	1.25	10.20	6.88
			After 12 weeks' incubation		
-	3.15	3.30	2.64	21.59	5.96
-	9.33	9.30	1.44	11.75	6.45
15	12.44	12.00	2.08	17.00	6.35
5	15.26	15.07	4.11	33.65	6.01
1	20.55	20.58	8.20	67.15	5.42
0.333	27.77	27.77	10.08	82.57	4.81
0.059	46.62	46.61	10.37	85.03	4.61
0.029	53.09	53.00	8.11	66.51	5.33
0.000	54.30	54.38	2.45	20.18	6.33
-	100.00	99.89	1.60	13.13	6.74

moisture tension of 0.059 atmospheres. It may also be noticed that oxidation occurred regardless of the moisture content or tension of the soil. It should be mentioned that the maximum amount of oxidation occurred at moisture contents wetter than field capacity.

It may be seen in Tables 4 and 5 that the two soils from eastern Kansas differ rather widely in their ability to oxidize sulfur.

Table 4. Sulfur oxidation and pH in a silty clay after a 3 week incubation period at various soil moisture levels.

Soil moisture tension in atm.	Percent moisture at start	Percent moisture at end	M.E. of sulfate /100 gms soil	Percent sulfur oxidized	pH
15	29.57	29.70	3.78	31.02	6.05
5	42.49	42.38	4.40	36.05	5.75
1	45.31	45.24	5.60	45.90	5.30
0.333	49.83	49.75	6.25	51.20	5.00
0.059	51.85	51.73	7.27	59.60	4.52
0.029	63.05	63.00	7.93	65.04	4.31
0.000	75.32	75.21	1.74	14.23	6.15

Table 5. Sulfur oxidation and pH in a loamy sand after a 3 week incubation period at various soil moisture levels.

Soil moisture tension in atm.	Percent moisture at start	Percent moisture at end	M.E. of sulfate /100 gms soil	Percent sulfur oxidized	pH
15	10.00	10.00	0.30	2.48	7.30
5	12.63	12.54	1.02	8.34	7.13
1	16.52	16.34	1.46	11.99	6.87
0.333	19.58	19.41	1.86	15.24	6.71
0.059	25.25	25.16	2.13	17.50	6.42
0.029	30.66	30.51	2.37	19.41	6.30
0.000	34.30	34.20	0.16	1.33	7.42

As the moisture content increased the amount of sulfur oxidized increased correspondingly. The maximum amount of oxidation occurred in both the loamy sand and silty clay soils at 0.029 athospheres or 30 centimeters tension. The maximum range of oxidation occurred at tensions wetter than field capacity.

Figure 2 shows the influence of soil moisture tension on sulfur oxidation in four soils during a 3 week incubation period. Soil moisture tensions are plotted on a logarithmic scale. Ulysses silt loam produces a maximum amount of oxidation at a tension of 0.029 atmospheres. The Fort Collins soil showed the greatest amount of sulfur oxidized at a tension of 0.059 atmospheres. The Ulysses soil oxidized 55.61 percent which may be compared to the low of 28.43 percent produced by the Fort Collins loam. The Fort Collins soil was unable to oxidize sulfur at 5 and 15 atmospheres and only a very small amount at 1 atmosphere. The Ulysses soil, however, was only unable to oxidize sulfur at 15 atmospheres. It may be noticed that the air dry, drier than 15 atmospheres, and the 1:1 ratio soil samples are not shown in Fig. 2. The Ulysses soil was able to oxidize small amounts of sulfur at these moisture contents. However, the Fort Collins loam showed no oxidation for the drier samples, and only a small amount at the wettest sample. It may also be noticed that at 0.029 athospheres the silty clay was able to oxidize 63.05 percent sulfur while the loamy sand was only able to show 19.41 percent, both being maximum for this period of incubation.

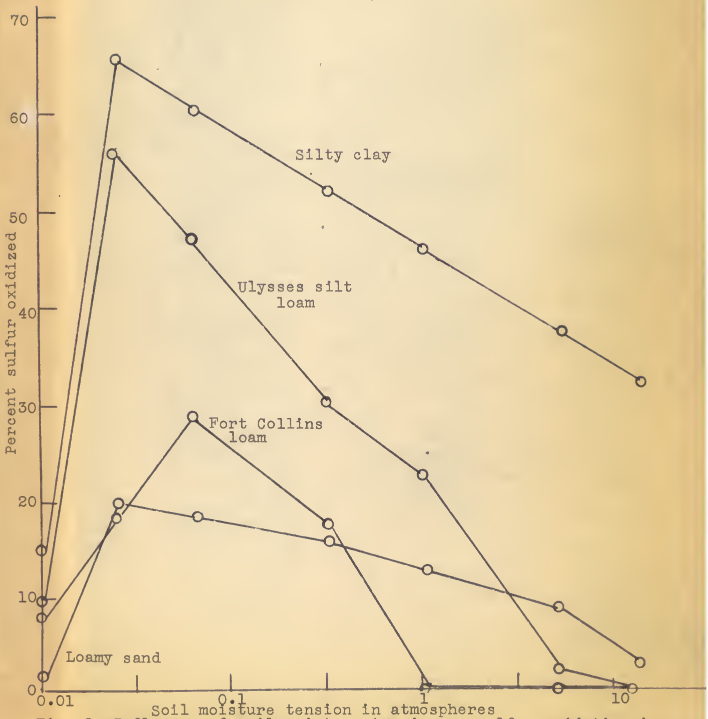

Fig. 2. Influence of soil moisture tension on sulfur oxidation in four soils during 3 weeks' incubation.

Figure 3 shows the influence of soil moisture tension on sulfur oxidation in Fort Collins loam and Ulysses silt loam during a 6 week incubation period. Again, the maximum amount of oxidation occurred in the Ulysses silt loam at a moisture tension of 0.029 athospheres, and the maximum in the Fort Collins loam at a moisture tension also of 0.029 atmospheres. The Ulysses silt loam was able to oxidize sulfur at all moisture contents ranging from the air dry samples to the wettest containing 99.85 percent moisture. The Fort Collins loam was still unable to oxidize sulfur in samples that had been subjected to 15 atmospheres and drier than 15 atmospheres tension. However, oxidation did occur in the air dry, and 5 atmosphere samples.

In Fig. 4 the effect of tension on the amount of sulfur oxidized during a 9 week period may be seen. It would appear from Fig. 4 that the Ulysses silt loam samples ranging in moisture tension from 1 atmosphere to 0.029 atmospheres had produced the maximum amount of oxidation possible. It may be noted that as the period of incubation increases the amount of sulfur oxidized also increases at all tensions and moisture contents for both soils. The Fort Collins loam, after 9 weeks of incubation, appears to be able to oxidize sulfur regardless of the moisture tension or content. It should be mentioned that the Ulysses silt loam reached this stage after 6 weeks of incubation. The maximum amount of sulfur was oxidized by the Fort Collins soil at a moisture tension of 0.059 atmospheres.

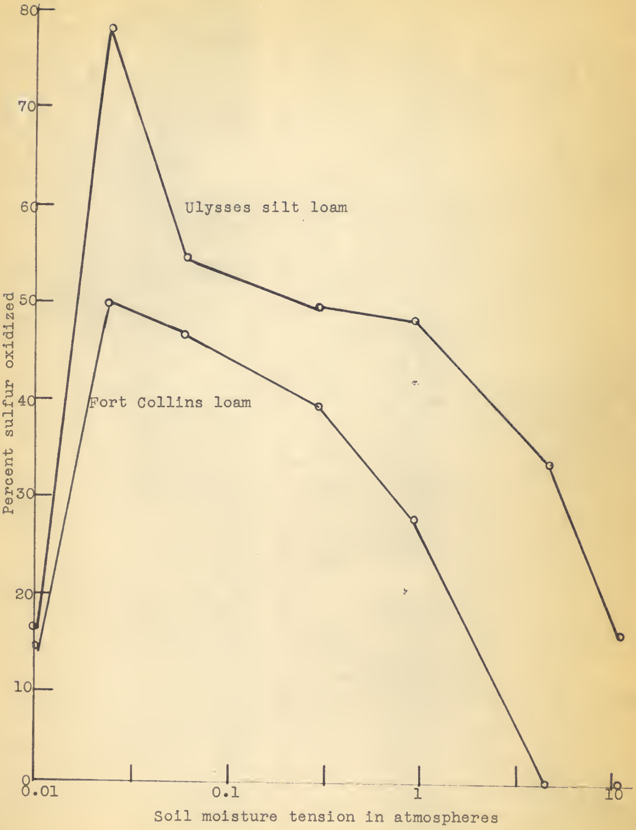

Fig. 3. Influence of soil moisture tension on sulfur oxidation in two soils during 6 weeks' incubation.

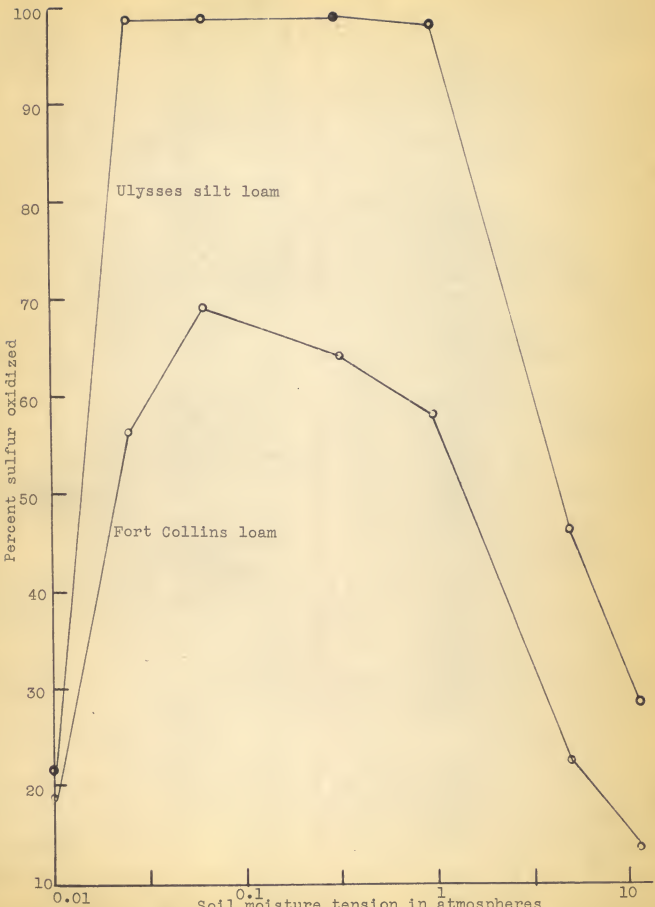

Fig. 4. Influence of soil moisture tension on sulfur oxidation in two soils during 9 weeks' incubation.

Figure 5 shows the results of 12 weeks of incubation in the Ulysses and Fort Collins soils. In regard to the Ulysses silt loam, it is a little difficult to state just what the optimum tension is, as tensions ranging from 1 to 0.029 athospheres produce the same results. The Fort Collins loam showed a maximum amount of oxidation at a moisture tension of 0.059 atmospheres. The maximum amount of sulfur oxidized by the Ulysses silt loam after 12 weeks of incubation was 100 percent while that of the Fort Collins soil was 85.05 percent. It would appear, from the past four figures, that the Ulysses silt loam oxidizes sulfur most quickly at 0.029 atmospheres, while the Fort Collins soil accomplishes this at 0.059 atmospheres with the exception of the 6 week incubation period. From the data given, it would appear that regardless of the moisture tension and sulfur content all the sulfur present eventually would become completely transformed into the sulfate form.

Figure 8 shows the influence of the soil moisture tension upon the hydrogen ion concentration in the four soils during a 3 week incubation period. The lowest pH showed by the Ulysses silt loam was 5.25 which corresponds to 55.61 percent, the maximum amount of sulfur oxidized. The Fort Collins loam showed the lowest pH of 6.30 at the maximum amount of sulfur oxidation of 28.43 percent. During this period of incubation the air dry, drier than 15 atmospheres, and the 1:1 ratio soil samples showed the highest pH for the lowest amount of sulfur oxidized in both soils from western Kansas. These figures are

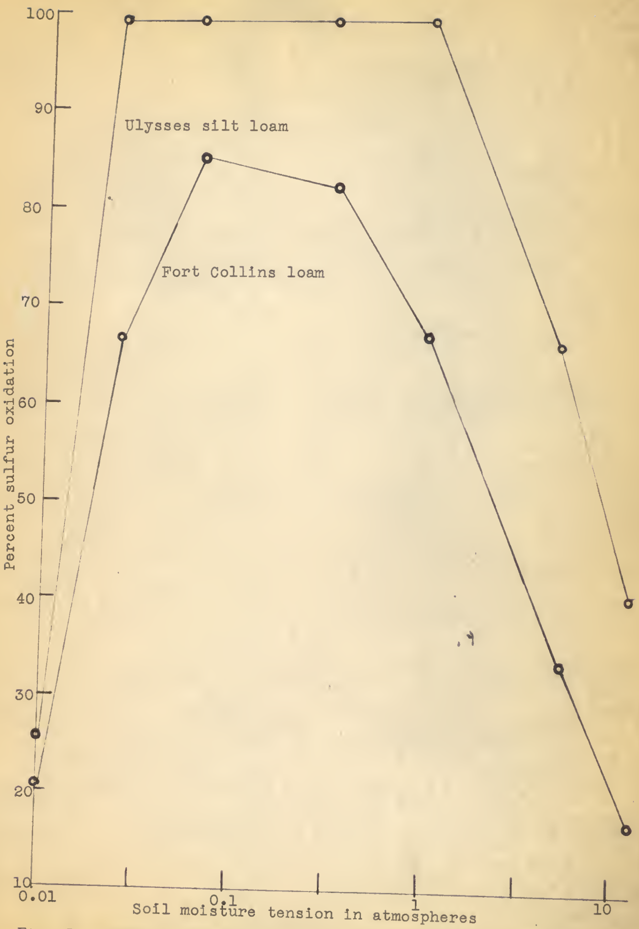

Fig. 5. Influence of soil moisture tension on sulfur oxidation in two soils during 12 weeks' incubation.

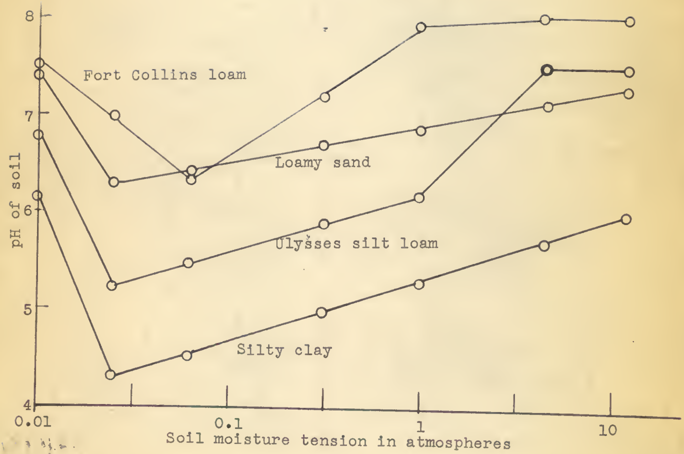

Fig. 8. Influence of soil moisture tension on pH in four soils during 3 weeks' incubation with sulfur present.

not indicated in Fig. 8 but may be seen in Tables 2 and 3. The lowest pH showed by the silty clay was 4.31 which occurred at the maximum amount of sulfur oxidation of 66.04 percent. The loamy sand on the other hand showed only 19.41 percent sulfur oxidized which corresponded to the low pH of 6.30. Both the silty clay and the loamy sand showed the maximum amount of oxidation and the lowest pH values at 0.029 atmospheres or 30 centimeters tension. Before incubation the silty clay had a pH of 6.23 and the loamy sand showed a value of 7.53.

Figure 9 shows the influence of soil moisture tension on pH in Fort Collins loam and Ulysses silt loam soils during 6 week incubation periods. The lowest pH values are shown by both soils at a tension of 0.029 atmospheres. These low pH's correspond to the maximum amount of sulfur oxidized in both soils. Again the air dry, drier than 15 atmospheres, and 1:1 ratio soil samples show the highest pH values which correspond to the lowest amounts of sulfur oxidized.

Figure 10 indicates the effect of soil moisture tension upon pH in the Fort Collins and Ulysses soil for a 9 week incubation period. The Fort Collins loam showed the lowest pH of 4.98 for 69.36 percent, the maximum amount of sulfur oxidized for this period of incubation. This low hydrogen ion concentration occurred at a moisture tension of 0.059 atmospheres. The Ulysses silt loam showed the lowest pH at the moisture tension of 0.029 atmospheres. This low pH corresponds to the maximum amount of sulfur oxidized for this period. The

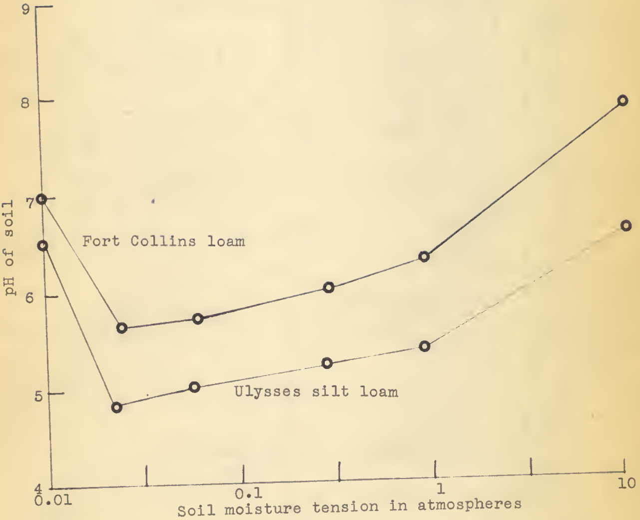

Fig. 9. Influence of soil moisture tension on pH in two soils during 6 weeks' incubation with sulfur present.

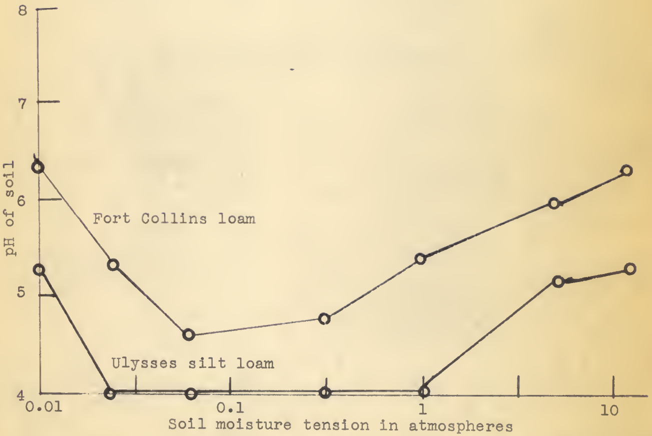

Fig. 10. Influence of soil moisture tension on pH in two soils during 9 weeks' incubation with sulfur present.

highest pH corresponded to the lowest amount of sulfur oxidized in the air dry soil, drier than 15 atmospheres and 1:1 ratio of soil samples.

Figure 11 may be compared with Fig. 5 in that the maximum amount of sulfur oxidized in the Ulysses silt loam corresponds to the minimum pH produced. This occurrence ranges in both Figs. 11 and 5 from 1 atmosphere to 0.029 atmospheres tension. The lowest pH of the Fort Collins loam for this period of incubation was 4.61 with the maximum amount of sulfur oxidation being 85.03 percent, both occurring at a moisture tension of 0.059 atmospheres. Again the highest pH corresponds to the lowest amount of sulfur oxidized in the two drier samples and the wettest of the group.

It can be seen from Figs. 6 and 7 that as the amount of oxidation increases the pH decreases. It should be mentioned that as the amount of moisture increases up to 0.333 atmospheres the pH decreases and the amount of sulfur oxidation increases. Before incubation the Ulysses silt loam had a pH of 7.53, while the Fort Collins soil had a pH of 8.03. It should be observed that the Fort Collins loam never showed a lower pH than 4.61, while the Ulysses silt loam was as low as 4.00. There is a tendency for initial increments of sulfur oxidation to influence soil pH to a greater extent than later increments.

It may be stated that the pH, moisture content, and amount of sulfur oxidized differ widely in the four soils used.

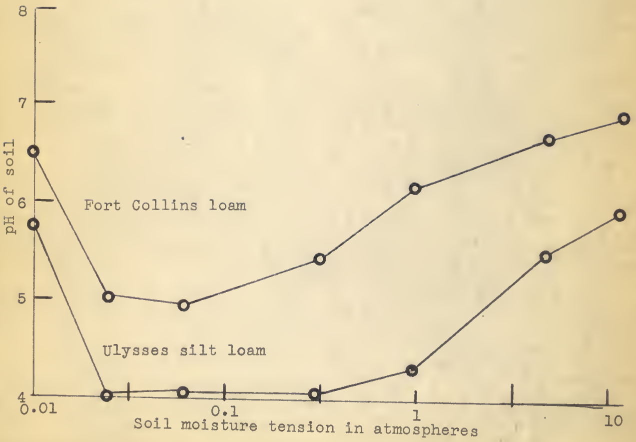

Fig. 11. Influence of soil moisture tension on pH in two soils during 12 weeks' incubation with sulfur present.

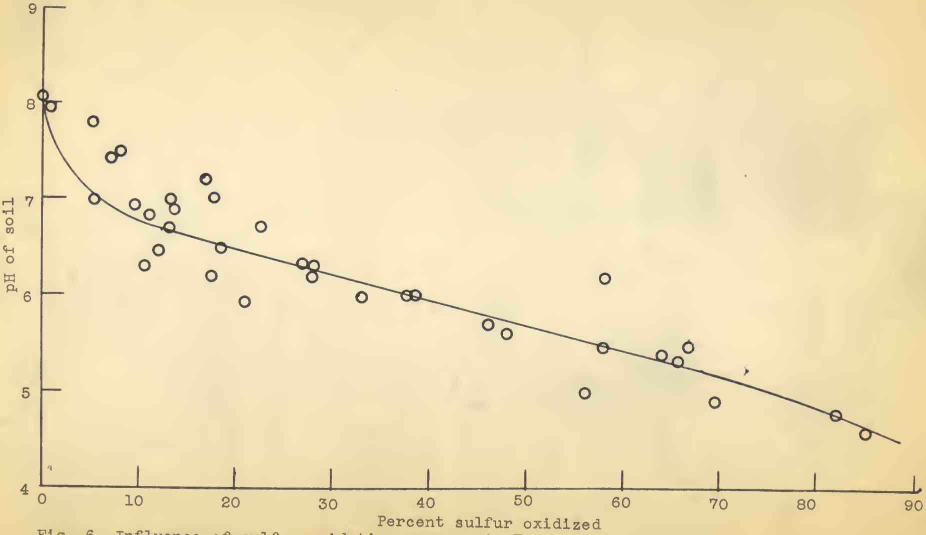

Fig. 6. Influence of sulfur oxidation upon pH in Fort Collins loam.

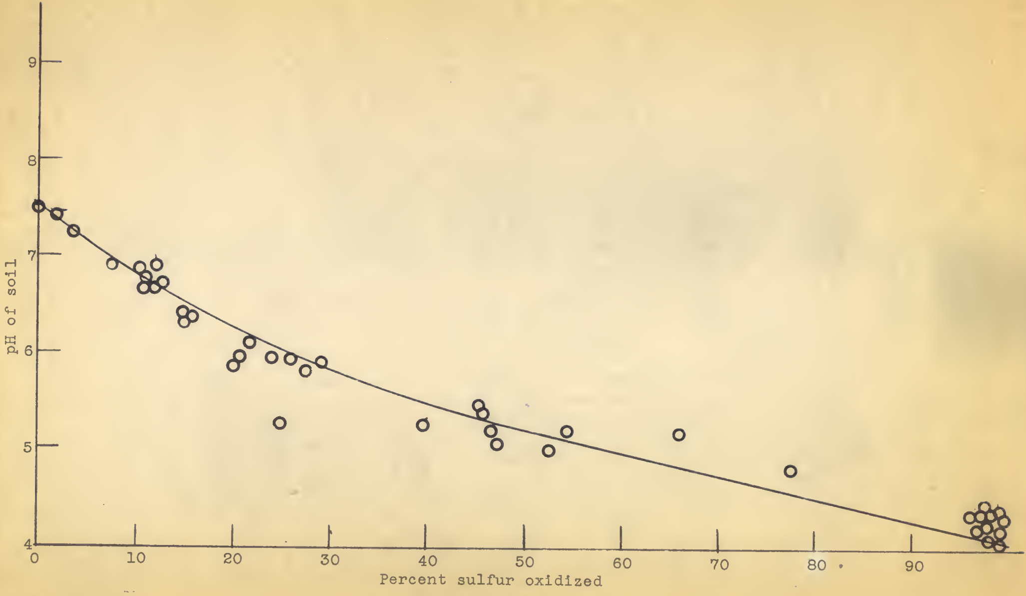

Fig. 7. Influence of sulfur oxidation upon the pH in Ulysses silt loam.

The Ulysses silt loam and the silty clay showed a greater decrease in pH, a lower moisture content and a greater amount of sulfur oxidized than the Fort Collins loam and the loamy sand.

Bacteria Found in Soils

It has been shown in the preceding sections that a great difference occurs between the oxidizing powers of Fort Collins loam and Ulysses silt loam. Therefore it was thought desirable to determine the kinds and amounts of sulfur oxidizing bacteria present in order to obtain a plausible reason for the obvious differences. No bacterial investigation was made of the two soils from eastern Kansas. The following discussion involves only the soils from western Kansas.

It may be seen from Table 6 that *Thiobacillus thiooxidans*, the highest sulfur oxidizer of the bacteria found, is prevalent in Ulysses silt loam in rather high amounts in samples containing sulfur and practically nonexistent in those without sulfur. *Thiobacillus thioparus* is present, but not to any great extent. The presence or absence of sulfur does not appear to affect the *Thiobacillus thioparus* to as great an extent as it does *Thiobacillus thiooxidans*. It can be seen that *Thiobacillus denitrificans* is affected the least of the three by the absence or presence of sulfur.

Table 6. Bacterial counts in Ulysses silt loam.

Bacterium found	Number of bacteria per gram of soil	
	Samples with sulfur	Samples without sulfur
Thiobacillus thiooxidans	200,000	10
Thiobacillus thioparus	20,000	1,000
Thiobacillus denitrificans	5,000	4,000

It can be seen in Table 7 that no *Thiobacillus thiooxidans* exists in the Fort Collins loam. This may be one of the reasons that this soil has such a low sulfur oxidizing power. A much greater number of *Thiobacillus thioparus* organisms exist in this soil than in the Ulysses silt loam. This organism is characterized by its slow oxidizing capacity. *Thiobacillus denitrificans* does not appear to be greatly affected by sulfur, but it is present in smaller amounts in the Fort Collins soil than in the Ulysses silt loam.

Table 7. Bacterial counts in Fort Collins loam.

Bacterium found	Number of bacteria per gram of soil	
	Samples with sulfur	Samples without sulfur
Thiobacillus thiooxidans	-	-
Thiobacillus thioparus	40,000	40
Thiobacillus denitrificans	3,000	2,500

It has been shown in the preceding sections that Ulysses silt loam has a lower original pH and also the pH decreases more rapidly than in the Fort Collins soil. Thiobacillus thiooxidans decreases the pH considerably when in thiosulfate liquid medium. Table 8 emphasizes this point.

Table 8. Influence of Thiobacillus thiooxidans upon the pH of thiosulfate liquid medium.

Incubation period in days	pH
0	6.30
2	6.00
4	5.83
6	5.41
8	4.98
10	4.54
12	4.23
14	3.92
16	3.51
18	3.29
21	3.00

In summary it may be stated that Thiobacillus denitrificans is prevalent in both soils regardless of the sulfur content. It may exist under both aerobic and anaerobic conditions. However, under anaerobic conditions it needs the presence of nitrates.

Thiobacillus thiooxidans is nonexistent in the Fort Collins soil, but very definitely exists in samples of Ulysses silt loam containing sulfur. It has been stated previously that this organism is almost nonexistent in soils that have not been enriched with sulfur.

Thiobacillus thioparus exists in both soils but is present in greater amounts in the Fort Collins soil, the lower sulfur oxidizing one of the two.

It should be mentioned again that one of the reasons that the two soils differ so radically may be their bacterial content, specifically their *Thiobacillus thiooxidans* content.

DISCUSSION

Waksman (40) makes the statement that one of the conditions in the soil that is favorable to the oxidation of sulfur is to have a moisture holding capacity of 50 percent. The data that have been presented in this paper indicate that the maximum amount of oxidation occurs in the moisture tension range of 1 to 0.029 atmospheres. This range is considerably wetter than the Waksman figure.

It may be noticed that the Ulysses silt loam showed a maximum amount of oxidation in the 9 and 12 week incubation periods that ranged from a moisture tension of 1 to 0.029 atmospheres. Similar results were obtained with the other three soils. With three of the soils the oxidation increased as the soil moisture tension became less until a tension of 30 centimeters was reached. With the fourth soil, oxidation was better at 60 centimeters tension than at 30 centimeters. It would therefore appear that conditions favoring the oxidation of sulfur in soils range from a moisture tension of 1 to

0.029 atmospheres and are not as dry as 50 percent of the moisture holding capacity of a soil as supposed by Waksman (40). It should be mentioned that this was the case in a silt loam, a loam, a sand, and a clay. One might also expect that soil water is more available to higher plants in the moisture range wetter than field capacity rather than drier. It is also likely that its availability becomes greater as the tension of water in the soil becomes less, so long as adequate aeration is maintained.

The laboratory results indicate that if samples were allowed to incubate long enough and range in moisture tension from 1 to 0.029 atmospheres, all the elementary sulfur present would be oxidized to the sulfate form. It also may be concluded that eventually, regardless of the moisture tension and elementary sulfur present, all this sulfur would be oxidized to the sulfate form.

It should be mentioned again that the field results coincide with the laboratory results in that the two soils from western Kansas vary rather radically in their ability to oxidize elementary sulfur to the sulfate form. Ulysses silt loam oxidizes the elementary sulfur to the sulfate form most rapidly at a moisture tension of 0.029 atmospheres, while the Fort Collins loam accomplishes this at 0.059 atmospheres.

It may be observed that the maximum oxidation of these two soils occurs in the Ulysses silt loam, probably because of its <u>Thiobacillus</u> <u>thiooxidans</u> content. The Fort Collins

loam, of course, will eventually reach maximum sulfur oxidation, but more slowly than the Ulysses silt loam. This difference may be due to the bacterial content of the two soils. Just why Thiobacillus thiooxidans is nonexistent in the Fort Collins loam is unknown. Perhaps the high original pH affects this organism to the extent that it cannot exist.

According to Bergey's manual (5), the activity of Thiobacillus thiooxidans is limited considerably by a pH above 6. The original pH of the Ulysses silt loam was 7.53, as compared to a high pH of 8 showed by the Fort Collins soil. The latter might have been high enough to not only retard activity completely but kill the organisms as well. It should be stated that as the pH decreased in the Ulysses silt loam, the amount of oxidation was readily increased. The Thiobacillus thiooxidans organism may have existed in the Ulysses silt loam in a dormant state and as the pH was decreased due to the addition of sulfur, its activity increased correspondingly. It should be noticed that the Fort Collins soil showed a slower decrease in pH than the Ulysses silt loam. As the Thiobacillus thiooxidans is the fastest sulfur oxidizer of the three organisms found, and as the Ulysses silt loam showed a greater and more rapid oxidation, the bacterial content may be the major cause of the great and obvious difference between the two soils.

Eventually the air dry samples would probably reach a maximum amount of oxidation without the benefit of the Thio-

bacillus thiooxidans, however, the length of time necessary to achieve this result would be great. The bacteria are responsible for the major portion of the sulfur oxidized, and it would appear that the presence of Thiobacillus thiooxidans would enhance the sulfur oxidizing power of a soil.

The agricultural significance of these data is great. It would seem that sulfur oxidation in irrigated soils could be brought about quickly if the soils were maintained at a high moisture content. Soils that are maintained wetter than field capacity will have much more rapid rates of sulfur oxidation than those that are allowed to dry to near the wilting point. With dry-land soils, sulfur oxidation will take place much more rapidly during wet seasons than during dry. As Thiobacillus thiooxidans is such a great sulfur oxidizer it might be wise to inoculate soils with this organism that show a deficiency in sulfate. In fact, in the areas of the country where sulfur deficiencies are known to be present, it may be possible to remedy the condition by the inoculation of all three sulfur oxidizing bacteria previously mentioned.

SUMMARY

An experiment was designed in order to determine the effect of soil moisture upon the amount of sulfur oxidized in soils to which elementary sulfur had been added. The problem of sulfur oxidation is important in various parts of the country

where a sulfur deficiency is present and in arid regions where it may be desirable to acidify soils. Four soils were used in this experiment, two, Fort Collins loam and Ulysses silt loam, from western Kansas and a loamy sand and a silty clay from eastern Kansas.

Ulysses silt loam, the loamy sand and the silty clay oxidized sulfur to the sulfate form most rapidly at a moisture tension of 0.029 atmospheres, while the Fort Collins loam accomplished this at 0.059 atmospheres. In each case the amount of sulfur oxidized became less as the soil moisture tension was increased above these tensions. As the amount of sulfur oxidized increased the pH decreased. The maximum amount of oxidation in all cases corresponded to the minimum pH values and the minimum amount of sulfur oxidation in all instances corresponded to the maximum pH values.

It was found that the two soils from western Kansas differed rather widely in their ability to oxidize sulfur. In order to determine the reasons for this difference, a bacterial study of both soils was made. It was found that one of the soils, Ulysses silt loam, contained the high sulfur oxidizer, Thiobacillus thiooxidans, while the other, Fort Collins loam, did not. It appears that the presence of this bacterium may be one of the causes for the radical difference.

Since the maximum amount of sulfur is oxidized at moisture tensions wetter than field capacity, one might expect that conditions are most favorable to the growth of higher

plants in this moisture range, so long as adequate aeration prevails. It also appears that soil moisture availability to plants decreases as moisture tension becomes greater.

It would appear that sulfur oxidation would occur quickly in irrigated soils kept at high moisture contents and in arid soils during the wetter seasons. It may prove beneficial to those parts of the country that are faced with the problem of sulfur deficiency to inoculate with the three sulfur oxidizing bacteria mentioned in this paper.

ACKNOWLEDGMENT

The writer wishes to express her sincere thanks to Dr. L. B. Olmstead of the U. S. Department of Agriculture and Dr. John Frazier of the Department of Botany and Plant Pathology for their assistance in conducting experiments. The writer also wishes to express her sincere thanks to her major instructor, Dr. R. V. Olson, for his helpful advice in conducting experiments and in the writing of the thesis.

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INFLUENCE OF SOIL MOISTURE AND SULFUR BACTERIA ON SULFUR OXIDATION IN FOUR KANSAS SOILS

by

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B. A., Hunter College
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1952

The experiment was designed in order to determine the effect of soil moisture upon the amount of sulfur oxidized in soils to which elementary sulfur had been added. The problem of sulfur oxidation is important in various parts of the country where a sulfur deficiency is present and in arid regions where it may be desirable to acidify soils. Four soils were used in this experiment; two, Fort Collins loam and Ulysses silt loam from western Kansas, and a sand and a clay from eastern Kansas.

Flours of sulfur were added to all soils at the rate of 4,000 pounds per acre of 0.2 gram per 100 gm of soil. The sulfur was sifted through a 100 mesh sieve.

In order to prepare sulfur treated soils at various moisture contents, 10 to 20 gram samples were first placed in rubber rings and soaked overnight. All samples were run in triplicate. The Leamer and Shaw tension table was used to adjust the moisture content of the soil to 0, 30, and 60 cm moisture tension. A porous ceramic plate placed in a pressure cooker operating on the principle of the pressure plate apparatus of Richards was used to obtain 1/3 and 1 atmosphere tensions. The Richards pressure-membrane apparatus was used for 5 and 15 atmospheres.

As it was not feasible to use the pressure-membrane apparatus for 30 atmospheres, the soil was adjusted to 15 atmospheres and allowed to dry in order to attempt to obtain the moisture retention that would occur at 30 atmospheres.

To determine whether or not sulfur could be oxidized under air dry conditions, 20 gm samples of air dry soil were used. In order to determine whether or not sulfur could be oxidized under anaerobic conditions 20 gm samples of soil were mixed with 20 ml of water.

Once the samples were adjusted to the soil moisture tensions mentioned, moisture contents were determined gravimetrically. The samples were then placed in stoppered bottles and allowed to incubate for 3-, 6-, 9-, and 12-week periods. Every other day the bottles were opened in order to insure aeration. At the end of a given period of incubation, pH, moisture content, and sulfate content were determined.

Ulysses silt loam, the sand and the clay oxidized sulfur to the sulfate form most rapidly at a moisture tension of 0.029 atmospheres, while the Fort Collins loam accomplished this at 0.059 atmospheres. In each case the amount of sulfur oxidized became less as the soil moisture tension was increased above these tensions and the soil became drier. At moisture tensions less than the above values, sulfur oxidation again became less, probably because of the development of anaerobic conditions. Some oxidation of sulfur occurred in all samples, regardless of the soil moisture content. It seems probable that even in air dry soils complete oxidation of sulfur would eventually occur.

As the amount of sulfur oxidized increased the pH decreased. The maximum amount of oxidation in all cases corresponded to the

minimum pH value.

It was found that the two soils from western Kansas differed rather widely in their ability to oxidize sulfur. In order to determine the reasons for this difference, a bacterial study of both soils was made. It was found that one of the soils, Ulysses silt loam, contained the high sulfur oxidizer, *Thiobacillus thiooxidans*, while the other, Fort Collins loam, did not. It appears that the presence of this bacterium may be one of the causes for the radical difference.

Since the maximum amount of sulfur is oxidized at moisture tensions wetter than field capacity, one might also expect that conditions are most favorable to the growth of higher plants in this moisture range, so long as adequate aeration prevails. It also appears that soil moisture availability to plants decreases as moisture tension becomes greater. It would appear that sulfur oxidation would occur quickly in irrigated soils kept at high moisture contents and in arid soils during the wetter seasons. It may prove beneficial to those parts of the country that are faced with the problem of sulfur deficiency to inoculate with the three sulfur oxidizing bacteria found in the two soils from western Kansas. These bacteria are as follows: *Thiobacillus thiooxidans*, *Thiobacillus thioparus* and *Thiobacillus denitrificans*.

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